Quantitation and Confirmation of Gabapentin

1 Introduction

Gabapentin is a drug used for its anticonvulsant and analgesic properties, however, the exact mechanism of action for gabapentin is not understood. Structurally, gabapentin resembles GABA (γ-amino butyric acid) but does not interact or interfere with $GABA_A$ or $GABA_B$ neurotransmitter sites within the body; furthermore, gabapentin crosses the blood-brain barrier unlike GABA. Gabapentin is not protein bound to any significant extent and is eliminated as the parent drug with a half-life of approximately five to seven hours.

2 Scope

This procedure allows for the quantitation and confirmation of gabapentin in blood. It may also be used for the confirmation of gabapentin in urine with proper validation.

3 Principle

Specimens are mixed with a deuterated analog internal standard, prepared for solid phase extraction using dilution, pH adjustment, and centrifugation. The specimens are extracted using Clean Screen® DAU solid phase extraction (SPE) cartridges and eluted from the cartridges using a solvent mixture. The eluent is then taken to dryness and reconstituted in a methanol water mixture and analyzed by liquid chromatography – electrospray – mass spectrometry (LC-ESI-MS).

4 Specimens

This procedure is validated for whole blood. Typically, 2 x 0.5 mL samples are analyzed; however, samples suspected to be above the procedure's linear range may be diluted before extraction.

5 Equipment/Materials/Reagents

a. 16 x 100 mm screw-top tubes with caps

b. 12 x 75 mm culture tubes with polypropylene snap-tops

c. Methanol (Optima grade or better)

d. Formic Acid (Puriss grade or better)

e. 0.1 M Sodium Phosphate Buffer (pH 6.0):
To a 500 mL volumetric flask, add 400 mL deionized water, 6.1 g sodium phosphate monobasic monohydrate, and 1.6 g sodium phosphate dibasic heptahydrate. Mix well to dissolve, verify that the pH is between 5.8 and 6.1; fill to the mark with deionized water. Store refrigerated in glass. Solution is stable for two months.

f. 0.1 M Hydrochloric Acid (0.1 M HCl):
To a 100 mL graduated cylinder, add 80 mL deionized water and 0.8 mL concentrated hydrochloric acid. Bring to 96 mL with deionized water and mix well; store at room temperature in glass. Solution is stable for six months.

g. Water (Deionized, 18 MΩ)

h. Centrifuge

i. Evaporator w/ Nitrogen

j. Vortex mixer

k. Methanol:Water (10:90 v:v):
To a 50 mL graduated cylinder, add 5 mL methanol (Optima grade) and 45 mL water Optima grade), mix well; store at room temperature in glass. Solution is stable for one year.

l. Elution Solvent [Methylene Chloride/Isopropanol/Ammonium Hydroxide (78:20:2)]:
To a 100 mL graduated cylinder, add 78 mL methylene chloride, 20 mL isopropanol, and 2 mL ammonium hydroxide, mix well. Solution is to be made and used on the same day.

m. Clean Screen DAU® SPE cartridges (regular flow) - 200 milligrams

n. Routine laboratory supplies, including disposable pipettes, wooden sticks, test tube racks, graduated cylinders, etc.

o. Liquid Chromatograph - Mass Spectrometer capable of 15,000 resolution.

p. HPLC Column (Xterra C-18 MS, 3.0 x 150 mm, 3.5 µm dp; or equivalent)

q. Mobile Phase A (0.1% Formic Acid in Acetonitrile):
To a 500 mL graduated cylinder, add 500 mL acetonitrile (Optima grade) and 0.5 mL formic acid, mix well; store at room temperature in glass. Solution is stable for two months.

r. Mobile Phase B (0.1% Formic Acid in Water)
To a 500 mL graduated cylinder, add 500 mL water (Optima grade) and 0.5 mL formic acid,

mix well; store at room temperature in glass. Solution is stable for two months.

6 Standards and Controls

a. d_{10}-Gabapentin Stock Standard (100 µg/mL):
Purchased from Cerilliant International or equivalent manufacturer. Storage conditions and stability determined by manufacturer.

b. Gabapentin Stock Standard (1.0 mg/mL):
Purchased from Cerilliant International and Lipomed or equivalent manufacturers. Storage conditions and stability determined by manufacturer.

c. Internal Standard Working Solution (100 µg/mL):
The Cerilliant, or equivalent manufacturer, deuterated stock solution is used as received. Store in glass at or below 0°C after opening; stable for at least six months.

d. High Calibration Working Solution (1.0 mg/mL):
Purchased from Cerilliant International or equivalent manufacturer. Storage conditions and stability determined by manufacturer.

e. Low Calibration Working Solution (100 µg/mL):
Add 0.5 mL of the 1.0 mg/mL Standard Stock Solution in a 5-mL volumetric flask and bring to the mark with Methanol. Store in glass at or below 0°C; stable for at least six months.

Table 1: Calibrator Preparation for Gabapentin (Add these amounts to 0.5 mL Negative Control Blood)

Calibrator Level (µg/mL)	High Cal WS (1 mg/mL) Volume (µL)	Low Cal WS (100 µg/mL) Volume (µL)
5	-	25
10	-	50
20	-	100
30	-	150
46	23	-
60	30	-
80	40	-
100	50	-

f. High Control Working Solution (1.0 mg/mL):
The Lipomed, or equivalent manufacturer, stock solution is used as received. Store in glass at or below 0°C after opening; stable for at least six months after opening.

g. Low Control Working Solution (100 µg/mL):
Add 0.5 mL of the 1.0 mg/mL Standard Stock Solution in a 5 mL volumetric flask and bring to the mark with Methanol. Store in glass at or below 0°C; stable for at least six months.

h. Negative Control Blood:
Purchased from Diagnostics Products Corporation, UTAK Laboratories, Inc., Cliniqa, or obtained in-house from a drug-free donor. Store refrigerated or frozen. Stability determined by manufacturer. A Negative Control Blood sample will be extracted and analyzed with every blood assay.

i. Quantitative Positive Control Blood:
This is normally prepared in-house as per the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101), but may be purchased from an appropriate vendor as needed. A Quantitative Positive Control Blood sample will be extracted in duplicate with every quantitative assay. The Quantitative Positive Controls will typically be prepared fresh from Control Working Solutions as described below:

1. Low Control (15 µg/mL):
Add 75 µL of the Low Control Working Solution to 0.5 mL of Negative Control Blood.

2. High Control (80 µg/mL):
Add 40 µL of the High Control Working Solution to 0.5 mL of Negative Control Blood.

j. Qualitative Positive Control Blood:
This is normally prepared in-house, but may be purchased from an appropriate vendor as needed. A Qualitative Positive Control Blood sample will be extracted and analyzed with every qualitative blood assay. The Qualitative Positive Control will typically be prepared fresh at any concentration above the assay's limit of detection from Control Working Solutions.

k. Column Performance Mix: Dilute 0.010 mL of the Internal Standard Working Solution with 0.090 mL of Methanol:Water (10:90 v:v). Prepare fresh.

7 Sampling

Not applicable.

8 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

a. To a properly labeled 16 x 100 mm screw-top tube, add 0.5 mL of specimen, calibrator or control. Prepare case specimens and positive controls in duplicate for quantitative analysis. Smaller volumes may be analyzed if required to ensure that the sample is within the linear range of the procedure. Case samples and positive controls do not need to be prepared in duplicate for qualitative analysis.

b. Add 30 µL of the Internal Standard Working Solution to each sample.

c. Bring all samples to approximately 5 mL with deionized water and vortex.

d. Add 2 mL of 0.1 M phosphate buffer to each sample and vortex. Allow to stand for 5 minutes.

e. Verify the pH of each sample is 6.0 ± 0.5.

f. Centrifuge for 10 minutes at 3500 rpm.

g. Prepare appropriately labeled SPE cartridges by conditioning each cartridge with 3 mL of methanol, 3 mL of deionized water, and 1 mL of 0.1 M phosphate buffer.

h. Load the samples onto the appropriate SPE cartridges.

i. Rinse the column with 2 mL of deionized water, 2 mL of 0.1 M HCl solution, and 3 mL of methanol.

j. Dry the cartridges under full vacuum for 90 seconds.

k. Apply 3 mL of freshly prepared Elution Solvent and collect eluent into appropriately labeled 12 x 75 mm test tubes.

l. Evaporate the eluent to dryness under nitrogen at 40^{o}C.

m. Reconstitute each sample in 200 µL Methanol:Water (10:90 v:v). Transfer 100 µL to an appropriately labeled autosampler vial.

n. Analyze 10 µL of each sample by LC/MS using the conditions below after verifying that the instrument is performing properly by analyzing the Column Performance Mix.

9 Instrumental Conditions

Appendix 2 contains an abbreviated version of the instrumental conditions in this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

9.1 Liquid Chromatograph Parameters

Mobile Phase Compositions		Flow Parameters			Column Parameters	
A: Acetonitrile with 0.1% Formic Acid		total flow	0.3 mL/min		type	C18-MS
		time (min)	%A	%B	length	150 mm
B: Water with 0.1% Formic Acid		0	10	90	internal diameter	3.0 mm
		5	10	90	particle size	3.5 µm
		20	90	10	temperature	30°C
Autosampler Parameters		30	90	10		
injection volume	10 µL	31	10	90		
temperature	15°C	37	10	90		
		total time	37 min			

9.2 Mass Spectral Parameters

ionization mode	electrospray (+)
scan mode	full scan, centroid
scan range	85 - 400 AMU
resolution	15,000
All source parameters are set through the instrument tuning process. See the Instrument Operations and Support Subunit SOP Manual for details.	

10 Decision Criteria

10.1 Batch Acceptance Criteria

No gabapentin should be detected in the Negative Control.

Gabapentin should be present in the Positive Control. Each Quantitative Positive Control will quantitate within ±20% of the target value. See the Guidelines for Toxicological Quantitations standard operating procedure (Tox 101) for more information.

10.2 Sample Acceptance Criteria

10.2.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Ion peaks are typically extracted at ±5 mmu. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

10.2.1.1 Retention Time

The retention time of the peak should be within ±5% of the retention time (relative or absolute, as appropriate) obtained from injection of an extracted Positive Control or extracted calibrator.

10.2.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Note: nonsensical signal to noise values may result from high resolution mass spectral data. Further, the baseline signal for the peak of interest should be at least ten-fold greater than that for any observed peak at similar retention time in a Negative Control or solvent blank injected just prior to the sample.

10.2.2 Mass Spectrometry

The M+1 for gabapentin in each sample should be 172.133 ±5 mmu.

The M+1 for d_{10}-gabapentin in each sample should be 182.196 ±5 mmu.

11 Calculations

Quantitation is performed by constructing a multi-point calibration curve based on the ratio of the area for the M+1 peak for the analyte to the internal standard. Ion traces are drawn at a 5 mmu mass tolerance. 1/x weighting is used. See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for acceptable practices in calculating quantitative results.

12 Measurement Uncertainty

The critical sources of measurement uncertainty in this procedure include:

- historical random uncertainty of repeated measurements
- accuracy of the pipette used to deliver the sample
- accuracy of the pipette used to deliver the calibrators

- uncertainty in the concentration of the calibration standards
- precision of the delivery of internal standard

When quantitative results are included in an FBI Laboratory report, the measurement uncertainty will be estimated and reported following the *Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements* standard operating procedure (CUQA 13). Information used to derive uncertainty measurements will be tracked in an electronic database.

13 Limitations

a. Linear range using 1/x weighting and a linear calibration model is 5 – 100 µg/mL.

b. Limit of Detection is 1 µg/mL

c.

	6 µg/mL	40 µg/mL	80 µg/mL
Bias (n=15)	-13.66	1.38	-2.96
Repeatability (n=15)	4.82	5.75	6.00
Intermediate Precision (n=15)	7.53	8.98	8.27

d. Interferences: None observed.

e. Carryover: For extracted negative control samples analyzed immediately following extracted 100 µg/ml calibrator samples, no carryover was observed.

f. Processed sample stability: the calculated difference between day 0 and day 1, 3, and 7 never exceeded ±10%.

14 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

15 References

Guidelines for Toxicological Quantitations (Tox 101); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

FBI Laboratory Practices for Validating Chemical Procedures; FBI Laboratory Operations Manual.

Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements (CUQA 13); FBI Laboratory Chemistry Unit Quality Assurance and Operations Manual.

Guidelines for Comparison of Mass Spectra (Tox 104); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

FBI Laboratory Chemistry Unit – Instrument Operation and Support Subunit SOP Manual.

FBI Laboratory Safety Manual.

Baselt, Randall; Disposition of Toxic Drugs and Chemicals in Man, 9th ed.; 2011

Neurontin®(Gabapentin) Product Monograph. Parke-Davis, Division of Pfizer Inc. NY, NY 10017; Apr2009.

Rev. #	Issue Date	History
0	08/20/15	New document.

Approval

Redacted - Signatures on File

Appendix 1: Abbreviated version of the Quantitation and Confirmation of Gabapentin Procedure for bench use

Redacted - Form on File

Appendix 2: Abbreviated version of the Instrumental Parameters for bench use

Redacted - Form on File